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KARYOTYPES OF THREE SPECIES OF *NOTOACMEA* (GASTROPODA: ACMAEIDAE)[1)]

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With Text-figures 1–5 and Table 1

Introduction

In recent years considerable amount of information has been accumulated on the chromosomes of Mollusca, and this point to the conservativeness of the chromosome numbers, which seems to be one of the important features of molluscan chromosomes. As for the chromosomes of marine prosobranchs, unfortunately, there is a scarcity of information, and what little information is available still mostly concerns only the numbers of the meiotic chromosomes. By the time mitosis has proceeded to metaphase the chromosomes have reached their most distinct and characteristic form. But the reports of the mitotic metaphase chromosomes of marine prosobranch molluscs are few, and are too incomplete or unclear to be used for comparative study.

The present study has two aims. The first is to re-examine previous descriptions of the chromosome number based on the squash or the older testis-section method, which has been reviewed and the results have been examined and revised in various groups of animals. The second is to determine the karyotype of the mitotic metaphase chromosomes as effectively as possible for future comparative study of molluscan karyology.

Materials and Methods

Karyological examinations were made on three species of *Notoacmea, N. concinna* (Lischke, 1868), *N. schrenkii* (Lischke, 1870), and *N. fuscoviridis* Teramachi, 1949. All specimens were collected from near the Seto Marine Biological Laboratory, Wakayama Pref., in October 1980, and were rendered for examination within a few days after collection.

For chromosomal study the materials were prepared by the usual air-dry method of Kligerman and Bloom (1977) with some modification: 1) The animals were put in 0.005–0.01 % colchicine solution for 6–12 hours before they were sacrificed.

1) Contributions from the Seto Marine Biological Laboratory, No. 682.

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2) Removed gill and testes were cut into small pieces and soaked in 0.075M KCl hypotonic solution for 20–30 minutes. 3) They were fixed in freshly mixed Carnoy's fixative (3:1 methyl alcohol acetic acid). 4) Tissues were then minced gently in 50 % acetic acid to prepare cell suspension. 5) The cell suspension was pipetted by a microhematocrit capillary tube and a drop of it was placed onto a clean slide on a heated slide warmer. 6) The suspension drop was immediately withdrawn back into the capillary tube so as to leave the cells on the periphery of the drop in a ring on the slide. 7) The cells left on the slide were dried and stained in 5 % Giemsa solution made up in 0.01M phosphate buffer at pH 7.0 for 10 to 30 minutes. 8) The stained slides were rinsed briefly and dried by applying warm air from a blower, and were then placed in two changes of xylene and mounted in Canada balsam.

The examination was done using Nikon and Olympus microscopes with a 100× (n.a. 1.25) oil immersion objective and 10–15× oculars. The following morphological features were considered for the comparisons among the three species: 1) Relative length of the chromosomes, percentage of the total length of the haploid set. 2) Arm ratio obtained by dividing the length of the short arm into that of the long arm of the chromosome. Nomenclature of chromosomes was adopted according to Levan et al. (1964), i.e.: arm ratio up to 1.7 as metacentrics; those up to 3.0 as submetacentrics; those up to 7.0 as subtelocentrics; and those over 7.0 as telocentrics.

Results

A total of 20 well spread metaphase plates were photographed and analysed: 7 from *N. concinna* (4 males), 7 from *N. schrenkii* (3 males and 2 females), and 6 from *N. fuscoviridis* (4 males).

The karyotypes of the three *Notoacmea* species examined in this study showed the chromosome number of $2n=20$ with very similar constitution except No. 3, 4, and 5. No. 3 and 5 are submeta- or subtelocentrics in *N. fuscoviridis* but meta- or submetacentrics in *N. concinna* and *N. schrenkii*. On the other hand, No. 4 is clearly metacentrics in *N. concinna* and *N. fuscoviridis* but submeta- or subtelocentrics in *N. schrenkii* with no overlap. Although the length of these chromosomes are similar in all three

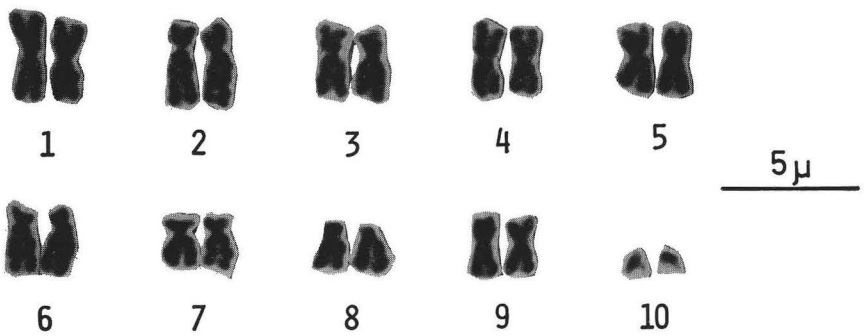

Fig. 1. Karyotype of *N. concinna* ($2n=20$).

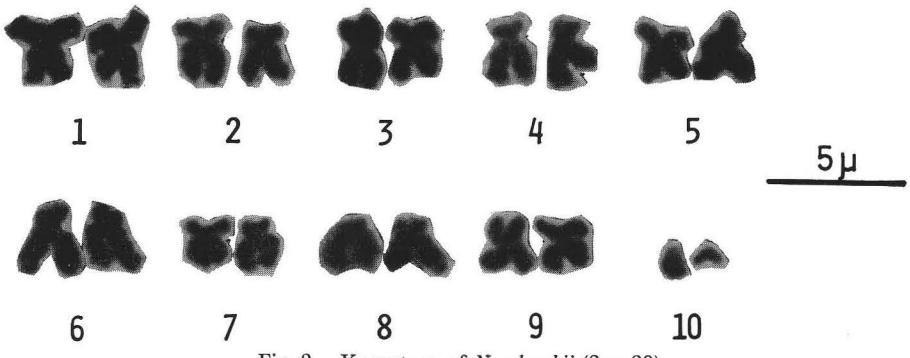

Fig. 2. Karyotype of *N. schrenkii* ($2n=20$).

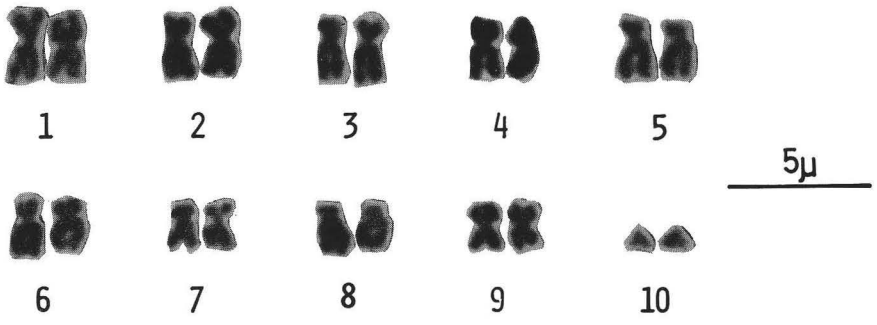

Fig. 3. Karyotype of *N. fuscoviridis* ($2n=20$).

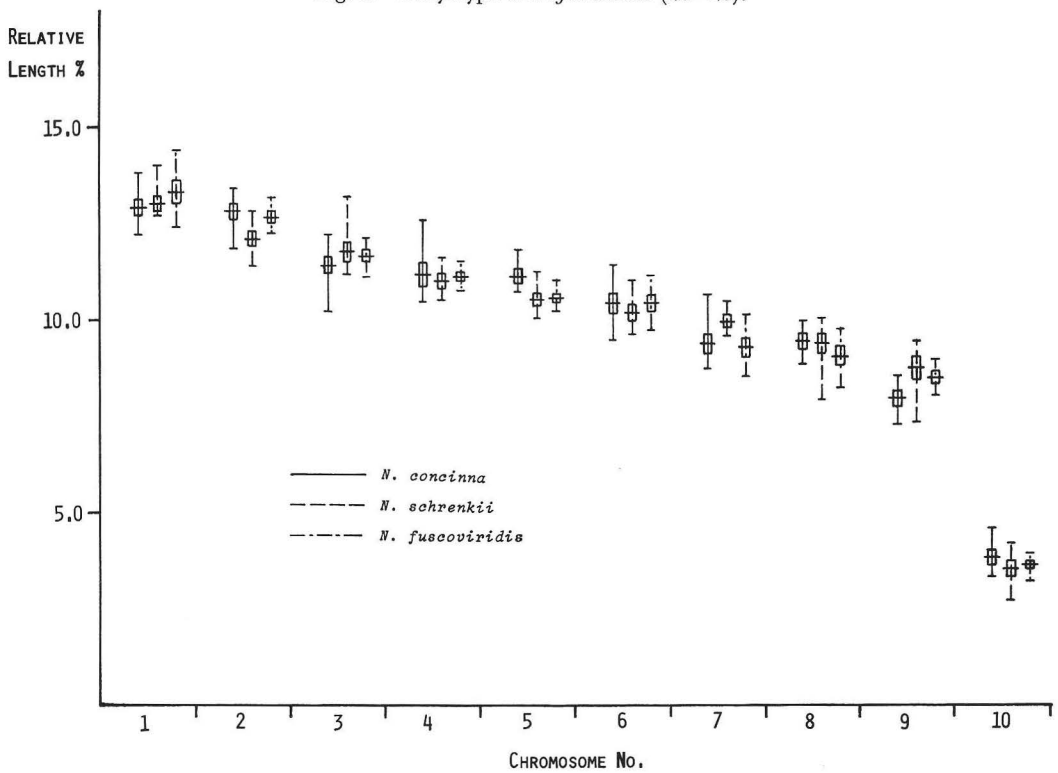

Fig. 4. Relative length (in % of length haploid chromosome set) of the chromosomes in *N. concinna*, *N. schrenkii*, and *N. fuscoviridis*. Indicated are mean (middle horizontal line), standard error (enclosed within boxes) and variation limits.

species, the position of the centromere is slightly different among the species (Figures 1–3). Other differences of the centromere positions of the chromosomes of the three species can be found, though they often lie within the limits of variation and it is very difficult to conclude that they may have some biological meanings or not.

The quantitative data of the measurements can be seen in Table 1 and Figures

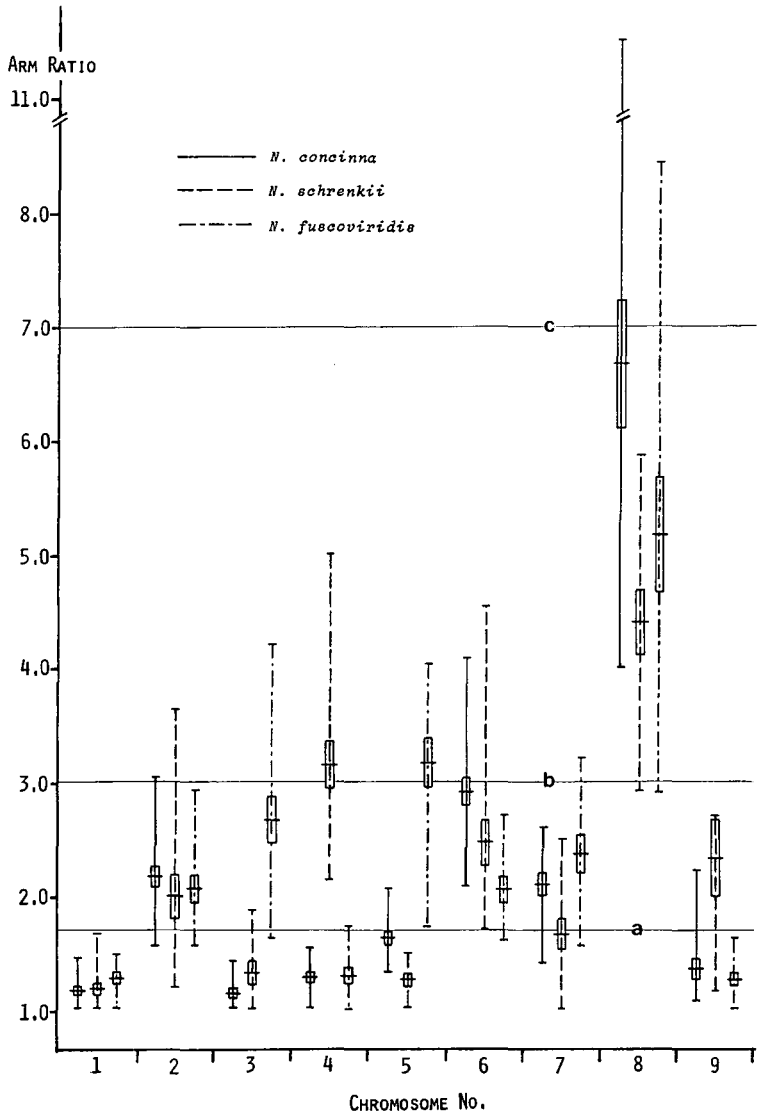

Fig. 5. Arm ratio (length of long arm divided by length of short arm) of the chromosomes of *N. concinna*, *N. schrenkii*, and *N. fuscoviridis* except No. 10 chromosomes which have no short arms. Mean, standard error and limits of variation are indicated as in Figure 4. Horizontal line (a) shows limit between metacentric and submetacentric chromosomes, line (b) between submetacentric and subtelocentric chromosomes, and line (c) between subtelocentric and telocentric chromosomes.

4–5. A pair of No. 10 was usually lightly stained and obviously small telocentrics without short arms. Other chromosomes were gradually decreasing in length from No. 1 to 9. The individual variations were sometimes large, even within one

Table 1. Chromosome measurement of *N. concinna*, *N. schrenkii*, and *N. fuscoviridis*.

Chrom.	R.L.(1)			A.R.(2)			Type(5)
	Max.	Min.(3)	$\bar{x}$—SE(4)	Max.	Min.	$\bar{x}$—SE	
N. concinna (7 plates)							
1	13.73	12.18	12.87±0.19	1.46	1.02	1.16±0.04	m
2	13.42	11.85	12.77±0.20	3.03	1.58	2.16±0.11	sm
3	12.17	10.22	11.36±0.24	1.41	1.01	1.15±0.03	m
4	12.55	10.45	11.15±0.29	1.54	1.02	1.28±0.04	m
5	11.80	10.67	11.07±0.15	2.06	1.32	1.62±0.05	m-sm
6	11.44	9.45	10.37±0.26	4.09	2.08	2.89±0.15	sm-st
7	10.60	8.72	9.34±0.23	2.59	1.42	2.14±0.10	sm
8	9.93	8.79	9.37±0.16	11.50	4.00	6.67±0.56	st-t
9	8.53	7.25	7.94±0.17	2.21	1.08	1.37±0.08	m
10	4.55	3.33	3.81±0.17	—	—	—	t
N. schrenkii (7 plates)							
1	14.04	12.72	12.99±0.18	1.67	1.03	1.19±0.05	m
2	12.80	11.43	12.11±0.17	3.60	1.21	2.01±0.18	sm
3	13.16	11.17	11.76±0.27	1.86	1.01	1.31±0.09	m
4	11.63	10.52	11.02±0.16	5.00	2.15	3.15±0.23	sm-st
5	11.25	10.07	10.53±0.14	1.51	1.03	1.26±0.04	m
6	10.95	9.57	10.16±0.18	4.55	1.72	2.46±0.21	sm-st
7	10.45	9.55	9.92±0.14	2.50	1.01	1.65±0.13	m-sm
8	9.98	7.90	9.37±0.26	5.86	2.91	4.39±0.29	st
9	9.37	7.29	8.73±0.28	2.69	1.18	2.33±0.33	sm
10	4.14	2.68	3.48±0.20	—	—	—	t
N. fuscoviridis (6 plates)							
1	14.38	12.41	13.32±0.29	1.52	1.01	1.28±0.05	m
2	13.15	12.26	12.67±0.13	2.92	1.58	2.07±0.12	sm
3	12.08	11.12	11.65±0.13	4.20	1.63	2.67±0.20	sm-st
4	11.51	10.75	11.10±0.11	1.76	1.02	1.30±0.07	m
5	10.95	10.2	10.53±0.11	4.04	1.77	3.18±0.20	sm-st
6	11.12	9.69	10.42±0.12	2.69	1.44	2.06±0.12	sm
7	10.11	8.47	9.27±0.24	3.20	1.41	2.37±0.19	sm
8	9.96	8.23	8.96±0.24	8.44	2.92	5.18±0.48	st-t
9	8.92	7.96	8.45±0.14	1.63	1.02	1.26±0.05	m
10	3.89	3.18	3.63±0.11	—	—	—	t

(1) R.L.: relative length expressed as % of length of haploid chromosome set.
(2) A.R.: arm ratio: length of long arm divided by length of short arm.
(3) Max.-Min.: range of variation.
(4) $\bar{x}$: mean, and SE: standard error.
(5) Type: m: metacentric (A.R. 1.0–1.7), sm: submetacentric (1.7–3.0), st: subtelocentric (3.0–7.0), t: telocentric (7.0 and above).

animal; this may be attributed to differences in the degree of condensation of the chromosomes in various cells.

Remarks

The chromosome number $2n=20$ revealed in the present study for the three species of genus *Notoacmea* is different from the results of previous work. Nishikawa (1962) reported 9 haploid chromosomes in spermatocyte of nine patellacean species including the present three species, based on the squash or the classical testis-sectioning method. It has been frequently indicated that these earlier methods lead to miscounting of the chromosome number, and this may be the reason for the difference.

The general conservativeness of the chromosome number among the molluscan taxa has been pointed out by some authors (Patterson 1969, Patterson and Burch 1978). The necessity and importance of examination of the mitotic metaphase chromosomes morphologically and quantitatively are also suggested by Patterson and Burch (1978) and Inaba (1979) with regard to taxonomic conservativeness. The karyotype is expected to serve even for the systemetic analysis on the generic and the species levels of molluscs. There is, however, little available information on whether the stable chromosomal condition might be retained only in a number of the chromosomes or even only in the morphology of each chromosome.

Patellacean limpets have been thought as one good example of the conservativeness of chromosome numbers among the molluscan taxa. The present study reveals that there is some possibility that chromosomal variability might exist in the superfamily Patellacea. The morphology of each chromosome apparently helps to identify the karyological characteristics of the genus *Notoacmea* and variations among the species. Even though the chromosome number might be identical, it has been indicated that karyological approach is effective even on the lower taxa of molluscs. Further comparative studies on the reported species and other members of the Patellacea will clarify aspects of the chromosomal changes and karyological divergence of the patellacean limpets and other prosobranch molluscs.

Summary

The chromosomes of the three *Notoacmea* species, *N. concinna, N. schrenkii,* and *N. fuscoviridis,* have been investigated. They have the same chromosome number of $2n=20$, which is different from previous results. The karyotypes of the three species have very similar constitutions except No. 3, 4, and 5 chromosomes whose positions of the centromere are slightly different among the species.

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